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Microbial Degradation of 2,4,5-T and Chlorinated Dioxins

Illinois Univ. at the Medical Center, Chicago

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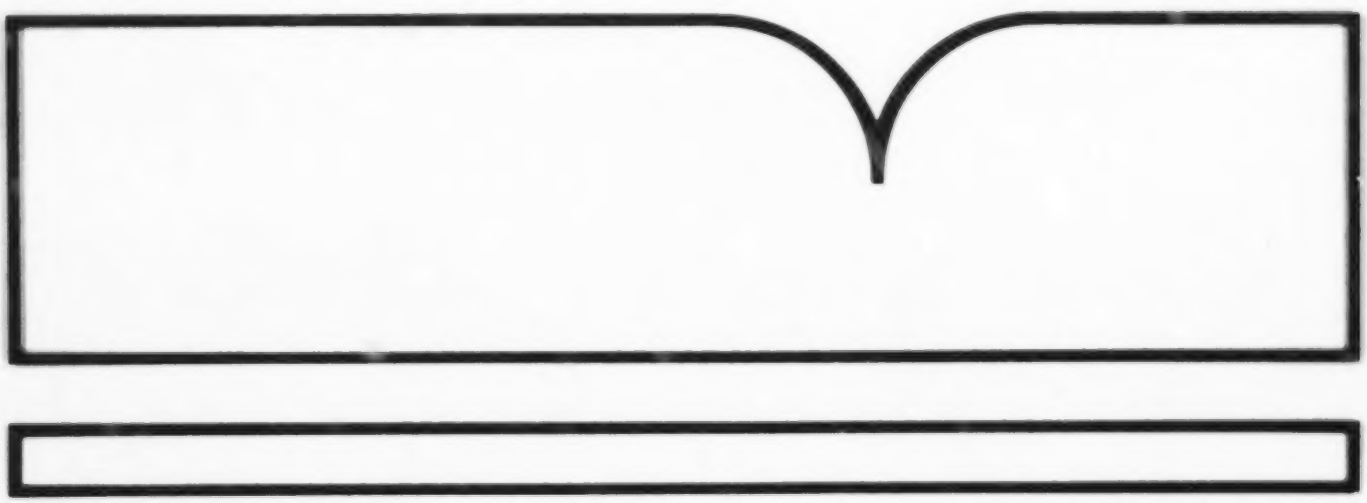

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16. ABSTRACT

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Extensive homology observed between plasmids points to the role of plasmid genes in the evolution and spread of degradative characters against toxic chemical compounds. A 1.3 kilobase pair DNA repeated sequence was found in strain AC1100. The specificity of this sequence to AC1100 suggests that this unique sequence may be a useful genetic probe of strain AC1100 and other novel Pseudomonas strains which are under consideration for deliberate release to aquatic and terrestrial environments.

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Microbial Degradation of 2,4,5-T and Chlorinated Dioxins

A. M. Chakrabarty

This report from the University of Illinois College of Medicine at Chicago covers three years of research under a Cooperative Agreement.

A pure culture of *Pseudomonas cepacia* strain AC1100, isolated from a chemostat enrichment culture experiment, is capable of growing on 2,4,5-T as its sole source of carbon and energy. Metabolic pathway studies indicate the activity of both constitutive and inducible enzymes. In laboratory experiments, soil contaminated with 2,4,5-T could be detoxified by AC1100 treatment, with the titer of AC1100 rapidly falling to nearly undetectable levels after the 2,4,5-T was substantially degraded.

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Introduction

This research brief describes the effort conducted under a cooperative agreement between the U.S. Environmental Protection Agency and the Department of Microbiology of the University of Illinois College of Medicine at Chicago. The research relates to the development and continued biochemical and genetic studies of a specific bacterial strain, *Pseudomonas cepacia* AC1100. These microorganisms can utilize a recalcitrant compound such as 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) as a sole source of carbon and energy, and can dechlorinate a variety of chlorophenols. Additionally, such a strain is shown to be highly effective in removing large quantities of 2,4,5-T from contaminated soil, thereby allowing the growth of plants that are normally sensitive to the presence of small quantities of 2,4,5-T. This work attempted to elucidate the organisms' metabolic pathways of degradation and gene recombination involved in the evolution of the strain.

The Role of Plasmids

Plasmids are known to play a major role in the biodegradation of a variety of complex organic compounds. However, only a few plasmids, such as pAC25, pWR1, and pAC31 are known to allow complete dechlorination of the compounds. The evolution of a chlorobenzoate degradative plasmid such as pAC25 specifying a number of new enzymes having maximal activity towards the chlorinated substrates, raises the interesting question as to how new degradative functions evolve in nature.

Several lines of evidence suggest that plasmids interact with one another to greatly extend the substrate range of plasmid-specified enzymes. Growth in a chemostat of 3-chlorobenzoate-degrading *Pseudomonas* species B13 with 4-chloro- or 3,5-dichlorobenzoate allows emergence of strains capable of degrading these compounds only when other *Pseudomonas* strains harboring the TOL plasmid are introduced into the chemostat. This project demonstrated that under such conditions, a part of the TOL plasmid undergoes transpostion on the chromosome to provide the broad substrate specific benzoate oxygenase function necessary for the conversion of chlorinated benzoates to the corresponding chlorocatechols.

Since growth with 3,5-dichlorobenzoate needs the participation of additional enzymes capable of dechlorinating the chlorolactone and chloromaleyl acetic acid, a segment of TOL containing the replication/incompatibility

genes and a segment of the chlorobenzoate plasmid pAC27 are recombined. This recombination gives rise to a separate plasmid, which then undergoes mutational divergence to generate new enzymatic activities for the dechlorination of the chlorinated intermediates. Thus, plasmids appear to play a vital role in nature as carriers of duplicated genes for their ultimate mutational divergence to generate new degradative functions.

Gene Regulation

Other useful information emerging from the studies of degradative plasmids relates to the extent of clustering of degradative genes on such plasmids. A physical map of the chlorobenzoate degradative plasmid pAC27 was constructed to demonstrate that the degradative genes are clustered within a single EcoR1 fragment of the plasmid. The cloned chlorobenzoate degradative genes, when transferred to *Escherichia coli*, are not expressed. The specificity of plasmid promoter sequences in *Pseudomonas* may help explain why, in spite of the transmissible nature of such plasmids, pseudomonads are the predominant scavengers in nature.

Based on the premise that plasmids evolve by recruitment of genes from other plasmids or from the chromosome, and that new genetic functions are acquired through mutational divergence of genes specifying analogous biological functions, laboratory culture conditions were developed which have yielded a strain of *Pseudomonas cepacia* (strain AC1100) that can utilize 2,4,5-T as a sole source of carbon and energy. Also, the AC1100 resting cell suspensions were found to be capable of oxidizing and dechlorinating a number of chlorophenols. Dehalogenation studies revealed that different halogen atoms on analogous molecules can lead to different efficiencies of dehalogenation. These results suggest that halogen atoms are removed in decreasing order F>Cl>Br>I, consistent with the general literature information on dehalogenation of halogenated aromatic molecules. The implication is that other halogenated compounds can be considered similarly biodegradable.

The regulation of the dehalogenating functions of AC1100 has been partially elucidated. The enzyme(s) for the conversion of 2,4,5-T to 2,4,5-TCP are constitutive, but the enzymes needed for 2,4,5-TCP degradation are inducible. Also, 2,4,5-TCP (or some metabolite), but not pentachlorophenol (PCP), can serve as an inducer.

Development of Strain AC1100

Pseudomonas cepacia AC1100 was developed in the laboratory using a chemostat. When the chemostat was switched over to 2,4,5-T as the sole source of carbon and energy, it was acting as a continuous enrichment culture and breeding ground for bacteria with the ability to rapidly metabolize 2,4,5-T and derive carbon and energy from it. Thus, as the genetic information necessary for the complete degradation of 2,4,5-T was evolving, growth on 2,4,5-T alone was the only selective pressure present. There was no selective pressure on this organism to evolve an effective means of regulating the 2,4,5-T degradative pathway in the presence of alternate carbon sources. Therefore, the manner by which the 2,4,5-T degradative pathway is regulated is thought to reflect the "evolutionary youth" of this organism.

Homologies

Plasmid pJP4, like other 2,4-D-degradative plasmids such as pJP1, pJP2, etc., was originally isolated from *Alcaligenes* sp., whereas both pAC25 and pWR1 were originally isolated from *Pseudomonas* sp. The question that arises is whether the difference in expression is due to (1) an inherent variation in the structural and regulatory genes on the 2 plasmids or (2) to a mismatch of the signal sequences present on plasmid pJP4 with the transcription-translation machinery of the new host, i.e., *Pseudomonas* sp. The second explanation seems plausible, since *Pseudomonas* plasmids are known to be expressed poorly in other hosts, particularly the enteric bacteria. However, the presence of unique structural features present in the plasmid pJP4 appears to indicate that a variation in the continuity of the structural and regulatory genes pertinent to 3-chlorobenzoic acid degradation is the major difference between pAC25 and pJP4.

Physical maps of pJP4 and pAC27 were constructed with extensive homology observed between the chlorobenzoate degradative plasmid pAC27 and the 2,4-D degradative plasmid pJP4 concerning the chlorocatechol-degradative genes and that between the same regions of pJP4 and the resident plasmids of the 2,4,5-T-degrading strain of *P. cepacia* AC1100. This points to the role of plasmid genes in the evolution and spread of degradative characters against toxic chemical compounds. Although this phenomenon is well-known in the evolution and dissimilation of antibiotic-resistance genes, no clear evidence for the occurrence of a similar trait in soil bacteria was available until now.

The structural studies performed in this work point to the important role of direct and inverted repeats in the genetic rearrangements necessary for rapid transition from growth on one substrate to that of a different substrate. The amplification of the structural genes in absence of a putative regulatory element is analogous to that observed in case of antibiotic resistance to allow the host microorganisms to withstand high and toxic concentrations of such antibiotics. Many toxic chemicals resemble antibiotics in terms of their inherent toxicity, both towards animals as well as microorganisms, and it is little wonder that microorganisms employ essentially identical tools, i.e., deletion, fusion, insertion, and other modes of genetic rearrangements, to help evolve new degradative functions. Continued studies of such processes are expected to provide unique insights regarding the mode of evolution of new biological functions in bacteria.

A DNA Repeated Sequence

In an effort to identify and localize AC1100 genes associated with 2,4,5-T degradation, transposon insertion mutagenesis was used to generate mutants blocked in the 2,4,5-T degradative pathway. A 1.3 kilobase pair DNA repeated sequence was found in the 2,4,5-T-degrading *Pseudomonas cepacia* strain AC1100. One copy of this sequence RS_{1100}-I was located near the chromosomal transposon 5 insertion site of a 2,4,5-T-negative mutant

designated PT-88. Thus, RS_{1100}-I appears to be closely associated with at least one 2,4,5-T gene and may have played a role in gene rearrangement. As judged by Southern hybridization analysis, the repeated sequence was not homologous to DNAs from several other *P. cepacia* DNAs and was absent in strains of *P. aeruginosa, P. putida, P. mendocina,* and other pesudomonads isolated from soil samples. The specificity of RS_{1100}-I to AC1100 suggests that this unique sequence may be a useful genetic probe of strain AC1100 and other novel *Pseudomonas* strains which are under consideration for deliberate release to aquatic and terrestrial environments.

Detoxication of Contaminated Soil

In laboratory experiments, soil previously contaminated with as much as 5,000 μg of 2,4,5-T/g of soil could be detoxified by AC1100 treatment, allowing the growth of plants sensitive to less than 10 μg 2,4,5-T/g of soil. Soil contaminated with as much as 20,000 μg of 2,4,5-T/g of soil showed greater than 90% degradation after six weekly AC1100 treatments. After 2,4,5-T has been substantially degraded in contaminated soil, the titer of AC1100 rapidly falls to nearly undetectable levels.

Publications

The following publications describe research supported by Cooperative Agreement, CR809666, between the U.S. Environmental Protection Agency and the University of Illinois College of Medicine at Chicago:

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7. Tomasek, P., B. Frantz, D. K. Chatterjee, and A. M. Chakrabarty. 1986. "Genetic and Molecular Basis of the Microbial Degradation of Herbicides and Pesticides," In: *Biotechnology for Solving Agricultural Problems*, Augustine, P. C., H. D. Danforth, and M. R. Bakst, eds., Marinus Nijhoff Publishers, Dordrecht, pp. 355-368.